THE CONCRESCENCE OF FOLLICLES IN THE HYPOTYPICAL OVARY.[1]

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In a preceding paper we have shown that lack of sufficient nourishment is the cause of a hypotypical condition of the ovaries. In one guinea pig we found in addition to the hypotypical condition another peculiarity of the ovaries, which is of considerable interest and which deserves a special description. In connection with the facts reported in the preceding paper it leads to some interesting conclusions as to the effect of underfeeding on the development of the ovarian stroma, and on the development of what in the guinea pig in certain respects corresponds to the interstitial gland of the rabbit and it may throw light on the origin of follicles containing more than one egg.

Female guinea pig No. 383 was obtained from Iowa. On January 12, 1917, it weighed 475 grams; on that date both lobes of the thyroid of this animal were removed. One thyroid lobe from another guinea pig, obtained from a different breeder and weighing 380 grams, was transplanted into the subcutaneous tissue of guinea pig No. 383. The transplanted thyroid was removed for microscopic study seventeen days later, January 29. At that time the guinea pig weighed 322 grams; it had therefore lost 32 per cent. of its original weight in a period of seventeen days. The transplanted thyroid was on the whole in a very good condition; we shall refer to this aspect of our findings in another connection. Here we are especially concerned with the condition of the sexual organs. The uterus shows low or medium cylindrical epithelium of the surface and glands with a very small number of mitoses in the surface epithelium. The mucosa is thin and fibrillar but very hyperemic. In the lumen of the uterus there are some erythrocytes. The ovaries are markedly hypotypical. They contain several atretic yellow bodies, the rem-

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nants of former corpora lutea. There are in the cortex a number of primordial follicles developing into small Graafian follicles and into follicles of small to medium size. But before they have hardly reached the latter size, a slow solution of the granulosa sets in and very soon the cavity of the follicles is lined by the theca interna.

The further course of the atresia of the follicles is, however, changed; the connective tissue does not as usual grow into the follicular cavity; instead the atretic follicles present themselves as small cysts. The ordinary follicles in various stages of connective tissue atresia are therefore lacking. There is, however, present a very large number of follicles in the last stage of atresia, a cavity with remnants of the zona pellucida, or merely a small cavity surrounded by a thick layer of the shrunken theca interna cells. By far the greater part of the ovaries consists of such follicles in the last stage of atresia; the proportion of such follicles is much greater in this animal than in normal ovaries which are in the period following the first week of ovulation and even larger than in the other hypotypical ovaries which we observed.

There are two further peculiarities noticeable in these ovaries, viz., (1) a relative decrease in the amount of fibrous tissue which surrounds the various follicles, and (2) the presence of numerous follicles with multiple eggs. While we observe around a number of growing or atretic follicles a fibrous membrane, the latter is always thin andit is absent around other follicles. Not rarely we see two adjoining follicles of small to medium size merely separated by several rows of relatively large theca interna cells; or we see small follicles close together, and not separated by fibrous tissue; at other places the theca internae of two quite atretic follicles press on each other, the theca cells of the one follicle surrounding directly the capillaries of the theca interna of the adjoining follicle. In other cases two or three shrunken egg cavities are enclosed in one mass of small theca interna cells.

If we compare the amount of fibrous tissue separating the follicles in this animal and in other animals with either normal or hypotypical ovaries, we find in the ovaries of other guinea pigs a larger amount of fibrous stroma separating the various follicles. This decrease in fibrous tissue made it possible to

differentiate microscopically the ovaries of this animal from those of other guinea pigs. There occurs in these ovaries a considerable number of follicles with two, three or even more eggs. We see in the most peripheral zone of the ovary two well preserved eggs of about the same size, but one slightly pressing upon the other and both surrounded by a single layer of granulosa cells. Somewhat further away from the tunica albuginea we see small follicles with two eggs of the same size, separated only imperfectly by a few flat granulosa cells. At other places we see follicles of small to medium size with a cavity. At the end of the cavity directed toward the center of the ovary there is a relatively large, well-preserved egg, surrounded by several rows of granulosa cells. At the side of the follicle directed toward the periphery of the ovary there is a very small, but well-preserved egg, in an earlier stage of development, surrounded by only one layer of granulosa cells. Here we have a combination of a primordial and of a small Graafian follicle. In other cases we see a small egg of a primordial follicle, pushing its way through the granulosa of the somewhat larger follicle and just reaching the cavity with the outer pole; the most frequent orientation of the two eggs is as follows: The larger one, surrounded by several rows of granulosa cells, is placed at the central pole of the cavity and the smaller one more toward the peripheral pole. But occasionally the order may be reversed, or the small egg may be at the lateral aspect of the follicle. In one case we found the egg of the primordial follicle in the suspensory ligament of the granulosa of the larger follicle. In still other cases we find a combination of a small egg at an early stage of its development, surrounded by one row of granulosa cells, the whole corresponding to a primordial follicle in the cavity of a small to medium follicle in which the granulosa and egg have already undergone degenerative changes. The degenerating ovum may still be surrounded by granulosa cells or the granulosa cells may have been dissolved. In other cases we find in a follicle of small to medium size two well developed eggs, both surrounded by several layers of granulosa cells. Similar combinations we find in follicles with three eggs. There may be combined a degenerated egg, a relatively large, preserved egg surrounded by several layers of granu-

losa, and an egg of a primordial follicle enveloped by one layer of granulosa. In a follicle in which we found a still larger number of eggs, the latter were well preserved and rather small, and situated in different parts of the granulosa. Of interest is also an observation which showed that a disintegrating egg may enter into direct contact with the theca interna of a nearby follicle of small to medium size.

These are the principal combinations of double or multiple eggs we observed in the ovaries of this animal, and both ovaries behaved in a similar manner. How shall we interpret this condition? The large number of follicles with two or more eggs which we found in both ovaries indicates clearly that we have to deal with a general condition of the organism affecting both ovaries equally and not with a local ovarian change. We may conclude that bioval or plurioval follicles may originate in either of the two following ways: (1) The young eggs in the tunica albuginea of the ovary remain united; connective tissue fails to grow between two or more eggs and to separate from each other the different eggs with the surrounding granulosa cells. The pictures of primordial follicles containing two eggs speak in favor of this interpretation. (2) But other follicles containing more than one egg must originate in a somewhat different manner. Very small follicles, especially primordial follicles, push their way into larger follicles, perhaps even into such follicles which are already in the process of degeneration. And this is probably by far the more frequent mode of origin. We find different stages in the junction of two follicles of different sizes, and in different stages of development. We can follow the pushing in of the smaller into the larger follicles. The fact that the thickness of the granulosa surrounding the different eggs in a follicle is in accordance with the character of the egg is only compatible with the view that two formerly distinct follicles effected a secondary union. In favor of this interpretation speaks also the observation that in the majority of cases the more undeveloped egg is situated toward the outer pole of the larger follicle. This is in accordance with the fact that the smaller a follicle is, the more peripheral is its situation. Occasionally, however, a primordial follicle may be pushed deeper into the cortical tissue

before it enters the wall of the larger follicle. In this case we might expect to find the smaller egg in the central part of the follicle and the larger one more toward the periphery. Whether also two small to medium follicles of similar size can unite to form one follicle with two eggs, we are unable to determine with certainty.

Essentially both modes of origin are in all probability based on the same condition, both being due to the same cause, namely, the relative inactivity of the connective tissue in these ovaries. We saw that in this animal the atresia of the follicles does not take its normal course, but that after disintegration of the follicles the connective tissue fails to grow into the cavity and that thus small follicular cysts syrrounded by theca interna are formed. We saw furthermore that the fibrous bands separating the various follicles are not so well developed as in the ovaries of other guinea pigs. It is therefore very probable that this deficiency in the connective tissue is at the bottom of this condition. The relative inactivity of the connective tissue, its failure to proliferate and to produce fibrous tissue, accounts for the imperfect atresia of the follicles and the imperfect separation of the follicles, and it is this inactivity of the connective tissue which is responsible for the occurrence of the follicles containing multiple eggs. In the first place, the connective tissue in some cases fails to grow properly between the very young eggs and their granulosa and to separate the two follicles; thus the first mode in the production of follicles with several eggs is brought about. It is due to a pathological persistence of an earlier stage in the development of follicles. And secondly the lack of development of strong fibrous tissue around a number of follicles, together with the pressure existing in the ovary as the result of the growth of young follicles, is responsible for the pushing of small follicles into larger ones. Thus there is no essential difference in the mode of origin of bioval or plurioval follicles at various stages of development.

The next question concerns the cause of this relative inactivity of the connective tissue. The study of the hypotypical follicles, which we reported in the preceding paper, throws light on this problem. We mentioned there that in cases in which the hypo-

typical condition was pronounced, there was noticeable a tendency to delay in the ingrowth of connective tissue into the follicular cavity; thus small cysts resulted. We interpreted this relative inactivity as due to the underfeeding which at last affects also the connective tissue. This condition represents a further stage in the chain of changes produced through underfeeding. This chain is as follows: (1) The maturation of follicles is suspended. (2) Abortion takes place in pregnant guinea pigs. (3) The granulosa cells of developing follicles are injured and are prematurely dissolved, but the relative strength of new formation on the one hand, and of destruction of granulosa cells on the other hand, shows such a balance in favor of new production that the development of follicles progresses so far that medium follicles are formed. (4) This balance becomes more unfavorable and only small and small to medium follicles develop; after this stage has been reached and the granulosa has been dissolved connective tissue grows in and fills the cavity and leads to a shrinking of the follicle. (5) In the last stage even the connective tissue becomes inactive. The ingrowth of connective tissue into the cavity does not take place. And the same process leads also in all probablity to an under-development of the fibrous bands separating the various follicles. Thus the separation of follicles becomes interfered with and the multiplicity of eggs in a follicle results.

It is, of course, possible that there are at work additional factors which favor such a process. It might be that in certain individuals the tendency of connective tissue to be inactive is greater than in others. It might also be that the number of the young follicles in early stages of development is greater in this animal and that thus a crowding of young follicles results. However, if such a difference exists between this and other ovaries, it is not very marked. Multiplicity of eggs in the ovarian follicles has been observed by us in several other ovaries of guinea pigs. At the time however when our previous observations were made we had not yet gained an undestanding of the connection between this change and the abnormal conditions which we found in this case. Ovarian follicles with several eggs occur also in other species, especially also in man. It would be

of interest to inquire whether in all cases the causes are the same. or whether different conditions may lead to the same result; In such an inquiry we have to keep in mind the possibility that a hypotypical condition of the ovaries might be a transitory state; that after having led to the production of follicles with multiple eggs, new follicles may again begin to develop normally, so that in the end nothing indicates that at one time such ovaries had been hypotypical.

This concrescence of follicles is somewhat analogous to a condition we find in other glands. In the thyroid of the guinea pig, as well as in the mammary gland of the mouse, we found occasionally structures which could only be explained as due to a concrescence of neighboring acini; due to the disappearance of the walls separating adjoining acini. We have therefore to deal with a phenomenon of a more general character, which under certain conditions may perhaps occur in all the glands.

There was in the ovaries of this guinea pig an additional feature which deserves special mention, viz., the very marked development of what corresponds to the interstitial gland in the rabbit. As we stated above, by far the greater part of this ovary consists of follicles in the last stage of atresia, follicles in which the shrunken cells of a well-developed theca interna surround one or several small cavities, the remnants of what were formerly follicular cavities. While we find in all hypotypical ovaries a relative preponderance of this kind of follicle, in the ovaries of this guinea pig this feature was more prominent than in the ovaries of the other animals. This is due to the following factors: (1) At the time of the thyroidectomy, the ovaries were in all probability relatively large, the animal weighing at that time 475 grams; thus space was available for the expansion of atretic follicles. (2) The follicles ceased to develop to medium or large size. Thus the pressure exerted by the larger follicles was eliminated, and a chance was given the atretic follicles to occupy the space otherwise occupied by large follicles. The slight development of fibrous bands around the follicles must have a similar effect diminishing pressure exerted on atretic follicles. We may therefore conclude that the structure analogous to the interstitial gland is prominent whenever the intra-

ovarian pressure is relatively slight. Under these circumstances the last stage of follicular atresia is preserved throughout a relatively long period of time. A similar condition we find in the ovaries of the guinea pig within the first week after ovulation, when the larger follicles have all become atretic.

Conversely we may conclude that the intraovarian pressure hastens the disappearance of the theca interna and of the other vestiges of atretic follicles. The pressure of the growing structures is stronger than the pressure of the resting and disappearing structures, and thus the life and growth in one part of the ovary hastens the disappearance of degenerating structures elsewhere. Not only is the disappearance of the shrunken theca internæ thus hastened, but also that of the fibrous tissue structures in the ovary. In our case it is uncertain whether the connective tissue had formerly grown into the follicles which are now in the last stage of atresia, this connective tissue having been absorbed subsequently, or whether in this animal the last stage of connective tissue atresia was reached directly from the cystic condition through absorption of the fluid in the follicular cavity, without a preceding ingrowth of fibrous tissue. In case the latter alternative should hold good the lack of ingrowth of connective tissue into atretic follicles would have been present for some time previous to the examination of the ovaries. In some atretic follicles we notice however some vestiges of fibrous tissue around the central cavity and it is therefore probable that in these cases the connective tissue had previously grown into the cavity after the solution of the granulosa. We also must consider the possibility that diminished intraovarian pressure may in itself influence the activity of the connective tissue.

Summary.

We describe the ovaries of a guinea pig in which the experimentally produced hypotypical condition was more advanced than in any of the other animals which we observed. In this case the underfeeding not only affected the activity of the granulosa, but also of the connective tissue. In consequence of a lowered activity of the connective tissue a concrescence of follicles takes place in numerous cases in both ovaries. A lowering of

intraovarian pressure in such ovaries leads to a relative preponderance of what corresponds to the interstitial gland in the ovaries of certain other species. The concrescence of ovarian follicles is analogous to the union of neighboring acini in the mammary gland and thyroid. It is a phenomenon which occurs probably in all or the majority of glandular structures.